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PATENT

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Date

Millicent A. Scarlett

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Martin A. Cheever and Mary L. Disis  
Application No. : 09/088,951  
Filed : June 2, 1998  
For : METHODS AND COMPOSITIONS TO GENERATE  
IMMUNITY IN HUMANS AGAINST SELF TUMOR  
ANTIGENS BY IMMUNIZATION WITH HOMOLOGOUS  
FOREIGN PROTEINS

Examiner : Karen A. Canella, Ph.D.

Art Unit : 1642

Docket No. : 920010.535

Commissioner for Patents  
Washington, DC 20231

DECLARATION UNDER 37 C.F.R. § 1.131

Commissioner:

We, Martin A. Cheever and Mary L. Disis, hereby declare as follows:

1. We are co-inventors of the subject matter described in the above-identified patent application (hereinafter referred to as "subject application").

2. All of the work described within this Declaration was performed in the United States, by ourselves or on our behalf and under our direction.

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3. We have reviewed our laboratory records, including the Exhibit submitted herewith, and readily conclude that methods, as claimed in the subject application, were conceived prior to October 1996. Further, due diligence was exercised from this time period until the invention was either actually reduced to practice, or until the filing of the subject application.

4. Prior to October 1996, we conceived the invention described in the subject application, i.e., that an immune response could be elicited or enhanced against self tumor antigens by immunization with homologous foreign proteins or portions thereof. In one embodiment of our invention, the self tumor antigen is prostatic acid phosphatase ("PAP"). Experiments from our laboratory confirmed our inventive concept by showing that immunization of rats with human PAP induced an immune response to rat PAP, whereas immunization of rats with rat PAP did not induce an immune response.


5. The following Exhibit (annexed hereto) represents a photocopy of laboratory notebook pages and a Western blot (all from prior to October 1996) kept in the regular course of business at the University of Washington. The dates have been removed from the copies submitted herewith. It our understanding, based on discussion with applicants' representatives, that this is a permissible Patent Office practice.

6. The Exhibit, which is a photocopy of several pages from laboratory notebooks and a Western blot (all from our laboratory), discloses the immunization of Fischer rats ("K") and Lewis rats ("CH") with PAP, e.g., rat PAP ("rPAP") or human PAP ("hPAP"). The Western blot shows that immunization of rats with hPAP elicited antibodies to both human and rat PAP.

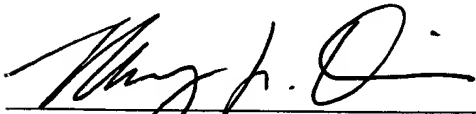
7. Included with the Exhibit is a compilation of data from additional experiments (also all prior to October 1996) from our laboratory. The data validates that rat PAP did not elicit an immune response in rats, but immunization of rats with human PAP induced an immune response to both rat and human PAP. Moreover, following immunization of rats with human PAP, the immunized rats could now be boosted with rat PAP.

8. In summary, upon review of our laboratory records, of which the above-cited pages are representative, we have concluded that, at least prior to October 1996, we had conceived of the methods as described and claimed within the subject application. Furthermore, our conception of the invention led to further research, diligently undertaken, resulting in an actual reduction to practice or in the filing of the subject application.

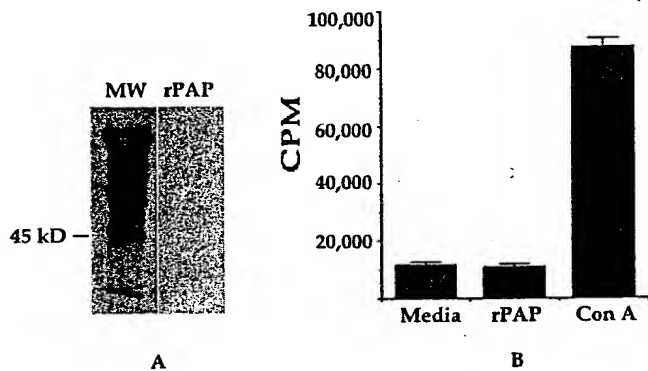
9. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

  
Martin A. Cheever

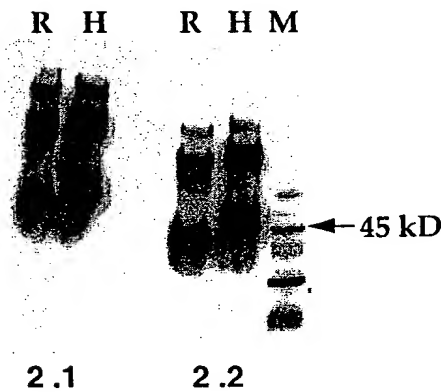
Dated this 5<sup>th</sup> day of April, 2002

  
Mary L. Disis

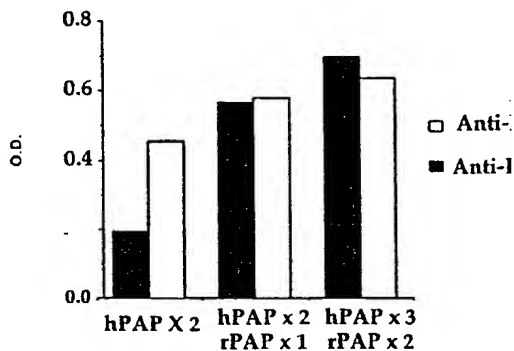
Dated this 14 day of April, 2002



No immunity to rat PAP was induced by immunization with whole rat PAP. Female Lewis rats were immunized with recombinant rat PAP (100  $\mu$ g) admixed with CFA and boosted twice with rat PAP plus IFA at three-week intervals. The antibody response against rat PAP (rPAP) was determined by western blot analysis (A) as described in Fig. 1. The T cell response was determined by a standard proliferation assay (B). Histopaque-purified splenic mononuclear cells ( $5 \times 10^5$ /ml) were incubated with either media, rat PAP (200  $\mu$ g/ml), or Con-A (5  $\mu$ g/ml) for 96 hours. Tritiated thymidine (1 uCi/well) was added to the culture for the last 8 hours. The thymidine uptake by T cells was determined by liquid scintillation counting (cpm).



IgG antibody responses to rat PAP could be induced by sequential immunization with hPAP and rPAP. Female Lewis rats were immunized with human PAP (100  $\mu$ g) plus CFA. They were boosted at three-week intervals with IFA plus human PAP (100  $\mu$ g) and with IFA plus rat PAP (100  $\mu$ g). Antibody responses to rat PAP or human PAP were determined by western blot analysis. Rat PAP (R) and human PAP (H) were run on a 10-15 % SDS-PAGE gel under reducing conditions. The blot was incubated with immune sera from two representative animals (2.1 and 2.2), followed by HRP-labeled goat anti-rat IgG antibody. The blot was developed with the ECL detection system. The molecular weight markers (M) are shown on the right.



IgG antibody responses to rat PAP after immunization with human PAP can be significantly boosted by subsequent injections with rat PAP. Female Lewis rats were immunized with human PAP (100  $\mu$ g) plus CFA. They were boosted sequentially at 3-week intervals with IFA plus human and rat PAP (hPAP x2, rPAP x1); or IFA plus human and rat PAP (hPAP x3, rPAP x2). Sera were obtained two weeks after each boosting. The amount of antibody to rat PAP (solid bars) or human PAP (open bars) in the immune sera was determined by an ELISA assay with 96-well plates pre-coated with either rat PAP or human PAP, respectively. The ELISA assay was developed with HRP-labeled goat anti-rat IgG antibody followed by HRP substrates, and the absorbance at 450 nm was determined.

R.K.C.

① ELISA<sup>+</sup> (Ginger.V.-)

100  $\mu$ g

1:1200

1:1600

1:2000

100%  
1:800  
40%  
1:1000  
57%

②

CHIV

1 Rev ♀

R. Throat

1 Rev ♂

Imm

④ Plates

→

③

Purification

Wednesday

Bent  
poor

KI & K~~IV~~

K~~IV~~

→ (✓ cells)

} cell

KV

→

V.A.S. & K~~IV~~

✓ Cut Ret -  
 ✓ Spin & suspend in  
 0.5% Triton / ARS

+ 1 ml of Bradford reagent

~ 100  $\mu$ l of sample + H<sub>2</sub>O

981 961 971.9  
 Mouse Ret  
 2x 4x 1x2

1.0  $\mu$ l of H<sub>2</sub>O or Blank

Blank 595  $\lambda$  0.00

Mouse 2x 0.257

Mouse 4x 0.474

Ret 1x 0.447

2x 0.679

0.2x 1.115

→ 1.95 mg/ml

→ 1.8 mg/ml

→ 6.8 mg/ml

→ 1.1

→ 8.8 mg/ml

1.9  
mg  
/ml

8 mg/ml

.066 = 1 mg

28 mg/ml

CTRL Ser → Free Zinn

CH II

Seisen  
&  
Boat

CH II

Tues

Grain-1's

Grain & Boat  
~  
QPS

CH III

(Fri)

Sami-1's

if CH II  
\* CH II Die

Spleen ul's  
+  
Ag  
+  
IL-2

→ Spleens

(+)

\* Risk-Ne

100% Buffers

CTRL

p39

Human

♀

♂

Nicely to Kent

Thursday

\*

KTII Case  
&  
Spleen

\*

Boat on Tues

CH II & CH III

KV

See & Saw for

GR  
PLS (2)  
Bain (4)

(012)

Freeze  
every 1h-

— X —

K II

Our by

}

CH12

CTR  
14w  
p35

Sera Tcell AdipK.s.  
Thyrog

p45  
p43

p45

(A)	140	1 .2 .3	3 flasks
(B)	140	1 .2 .3	3 flasks

CH12

CTR  
14w  
p35

Sera  
+  
13 sera

Sera on  
13 sera on

sample on

Groups } Sera  
          } Observe Key e specimens

Rik will run individuals

Image ~

CHI

- |   |             |     |                |
|---|-------------|-----|----------------|
| ① | → CTRL      | x 2 | 1              |
| ② | → Human PAA | x 4 | 2 <del>→</del> |
| ③ | → 37        | x 6 | 2              |
| ④ | → 45        | x 6 | 2              |
| ⑤ | → 43        | x 2 | → <del>2</del> |

1st 2mm

2nd 2mm

3rd 2mm

All ~~for~~

See on

1  
See Weikins

P35

P45

6

6

3 P35

3 P45

~~3~~

3 P100

from

Peptides

750/ug each

Bovine PAF 400ug

KV

① CTRL → 2 1 1KV 2mm

② Bovine PAF → 4

③ p39 → 6 ——— Bovine PAF

④ p45 → 6 ——— Bovine PAF

⑤ p43 → 2 ———

7000

\* Sera

\* Bovine

\* Do Sera from a Western

3rd Im

200pg each

Imm on

Bovine  
100  
100

Rev  
100  
100

Human  
100  
100

KIV

2nd-1/5 group

- ① → CTL
- ② → Bovine + Rev
- ③ → Bovine
- ④ → Human + Rev
- ⑤ → Human

1 Rev / group  
remains

1st Imm →

2nd Imm →

3rd Imm →

4th Imm →

} 1 Rev / group sacrificed

> 4 wks

7000

\* SACRIFICE OTHER ONE =  
Imm scan + GUSST 5020

Imm  
ant

\* V T cell results (Single 1 v 1)

\* WESTERN

	<u>Rev</u>		Rev 2			
①	✓	✓	✓	✓		
②	✓	✓	✓	✓	✓	✓
③	✓	✓	✓	✓	✓	✓
④	✓	✓	✓	✓	✓	✓
⑤	✓	✓	✓	✓	✓	✓
		10 gels	20 gels			

(KPI)

4 + 1 <sup>2nd</sup> <sup>1st</sup>  
group

✓

① → GM-CSF

② → Bovine PAD + GM

③ → Bovine PAD

④ → GM + Rat PAD

⑤ → Rat PAD only (4 Imm  
thru)

To Do

\* ✓ T cell results

\* All rats sacrificed on

\* Histology

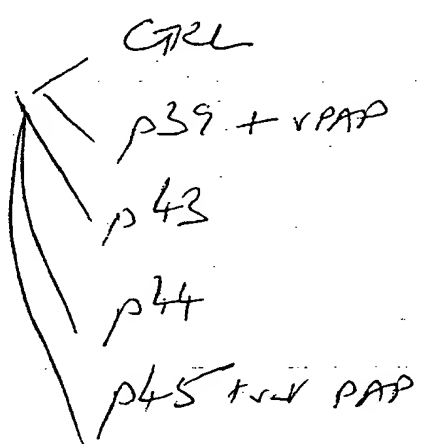
9 gels



~~Pept. des~~ ~~59~~ ~~43~~ ~~44~~ ~~45~~ ~~5~~ ~~2013~~ Pept. des 59 ✓ 43 ✓ 44 ✓ 45 ✓  
 Protein 400mg total

(LD) Reed 1st Imm & 1st A accidentally  
 2/group

Sgroup



1st Imm  
 2nd Imm

2 sides QC & 2 sides 2M  
 Pept. des + Protein [hooking test]

(To Do)

\* Immunize ✓  
 - Westkoppel ✓  
 ✓ Sera collect-  
 SERA on

(KI)

B Group



1st/2nd

(4th Imm)

① → PBS/LFA / IFA → 4th Imm all seq

② → p39 + 100mg/ml PAP

③ → p43

④ → p44

⑤ → N+5  
+ 100mg/ml PAP

⑥ → Rev PAP → Induced 6 K3

All rats  $\bar{x}$  Group 6 → SACRIFICED -

Ts do

\* ✓ Tcell results

Western

CR1 — 6 [6 gels]

1.1

11111

HMW

Human

Homogentz

Ref

1

111

LMW

2.1 & 2.2



HMW

Human

Homogentz

Ref

1

LMW

11111111

5/5/5